# Biomarkers in Periodontal Diagnosis: "What The Future Holds..."

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## Abstract

The ability to monitor health status, disease onset and progression, and treatment outcome through non-invasive means is a most desirable goal in health-care promotion and delivery. There are certain ground rules for this goal to be realized: specific biomarkers associated with a health or disease state, a non-invasive approach to detect and monitor the biomarkers, and the technologies to discriminate between and among the biomarkers. We in the present literature have tried to assess a pathway to achieve these goals using oral fluids as the diagnostic medium to analyse the health and/or disease status of individuals. As the "mirror of body", oral fluid is a perfect medium to explore regarding health and disease regulation.

**KEYWORDS:** Biomarkers, non-invasive, diagnostic.

## Introduction

A biomarker is a substance used to indicate a biologic state. It can be defined as, "A substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention[1]." Traditional biomarkers in medicine include heart rate, blood pressure, imaging (X-rays), and the prostate-specific screening antigen test (PSA) for prostate cancer.

As periodontitis is considered to be a multifactorial disease with no clear cut etiology, so its identification and early diagnosis becomes all the more difficult and important. So with the current objective the need for periodontal diagnostic tool which would provide adequate information for differential diagnosis, localization of disease, and severity of infection arises all the more. These diagnostics should not only aim at mere diagnosis of the underlying disease but should also serve as a basis for planning treatment and provide the means for assessing the effectiveness of periodontal therapy. Ironically, the current clinical diagnostic parameters that were introduced more than 50 years ago continue to function as the basic model for periodontal diagnosis in clinical practice today. These include probing pocket depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs that quantify alveolar bone levels[5] these traditional biomarkers were attributed with ease of use, cost-effectiveness, and relatively non-invasive.

A traditional biomarker for periodontal disease is bleeding on probing (BOP), supposedly the best disease predictor available today (Fig.1). According to Researchers there are many false positives associated with it, but the absence of BOP is considered a very precise negative predictor of disease activity[5], periodontal diagnosis is based upon subjective clinical examination procedures that may be time-consuming and poorly implemented by the operator. Many a time diagnosis is solely based upon

periodontal probing measurements due to time constraints, which lead to under diagnosis & inappropriate treatment and low rates of appropriate therapeutic intervention.

**Dilemmas in decision making and the way out**:-

- How can clinicians assess risk for periodontal disease?
- What are the useful laboratory and clinical methods for periodontal risk assessment?
- What can be achieved by controlling periodontal disease using a risk profile?

Risk factors are considered modifiers of disease activity. In association with host susceptibility and a variety of local and systemic conditions, they influence the initiation and progression of periodontitis and successive changes on biomarkers. Therefore, additional diagnostic and prognostic tests have been extensively sought to address these problems. Thus advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers.

Diagnostic tools to measure periodontal disease at the molecular, cellular, tissue, and clinical levels[2] are:
At molecular level activation of receptors for endotoxin: CD-14, toll like receptors occurs which can be diagnosed by PCR, DNA hybridization etc.
Similarly at tissue & cellular level; ELISA, Immunohistochemistry can be used. At clinical level periodontal probing, radiographs have always been there.

Chronic periodontitis is now considered a site-specific disease, but what has continued to puzzle periodontal researchers is the unpredictability of the disease at a patient and site-level. As a result, a flurry of research activity was underway in the 1990s to study the utility and value of individual biomarkers of periodontal disease activity. Researchers such as Chapple[3] thus created biomarkers that indicated the presence or absence of periodontal pathogens, gingival and periodontal inflammation, the host inflammatory-immune response to certain pathogenic species, and host tissue destruction. The biological media of choice included saliva, serum, subgingival plaque, tissue biopsies, and gingival crevicular fluid. As a result, and after many biomarkers and diagnostic tests were developed, a number of diagnostic kits became available. These kits included biomarkers within gingival crevicular fluid, but market research wasn't performed and the kits failed to reach the practice community. Chapple[3] reported problems as they were not user friendly, costly, time consuming, & complex to perform & understand.

Saliva was then looked at as another choice for an appropriate diagnostic medium. The challenges are enormous, because it still isn't known what the exact phenomena are that trigger the cascade of events leading to tissue destruction, and a biomarker needs to predict disease onset and progression. It's possible that the markers associated with inflammation are not the ones responsible for initiating the disease process. It's also possible that markers that characterize the inflammatory process in gingivitis are different from those that develop in periodontitis.

A biomarker is an objective measure that can be evaluated and confirmed and not only any particular substance as was stated in definition. Oral fluid biomarkers that have been studied for periodontal diagnosis include proteins of host origin (e.g. immunoglobulins), phenotypic markers, host cells (e.g., PMNs), hormones, bacteria and bacterial products, ions, and volatile compounds. Since periodontitis is a multifactorial disease that includes initiation by bacteria and host interaction, it's unlikely that a single biomarker will be able to predict periodontal disease activity. So combinations could be tried.

**CURRENT TRENDS:**

Recent advances in the use of biomarker based diagnostics for disease activity include mediators that are released into GCF & saliva can be broadly grouped according to their sources as grouped as[2,4]

1) **Microbial plaque**: Endotoxins (lipopolysachrides), Enzymes, Metabolic end products, DNA probes, Cultures of putative periodontal pathogens.
2) **Host derived** :- 1L-β, Aspartate, Aminotransferase, Transferase, Matrix proteins, Lactoferrin, Lysozyme etc…
3) **Connective tissue breakdown products**: Collegen-telopeptides, Osteocalcin, Proteoglycans, Breakdown products, Fibronectin fragments.
4) **Inflammatory mediators:** Complement, Cytokines, Interleukins, Tumor necrosis factor-α, Interferon-α, Antibacterial Antibodies IgG, IgM, IgA, Substance P, Prostaglandin $E_2$, Acute-phase proteins, transferrin, C-reactive protein.

Also Specific salivary biomarkers for periodontal disease, which are considered to be the mirror of the body are enlisted below [5] (Table 1).
All the enzymes released due to inflammatory process may or may not be truly associated with the disease activity. It is therefore very important to show that a potential marker has a true association with periodontal disease activity which is independent of and stronger than any association it may have with gingival inflammation. This is most clearly shown in comparisons of true and false positive and negative sites in respect of confirmed attachment loss which are used to compute the sensitivity, specificity and positive and negative predictive values of each markers.

| Proteomic Biomarkers | Genetic Biomarkers | Microbial Biomarkers | Other biomarkers |
|---|---|---|---|
| **Cystatins, α-glucosidase, Acid phosphatise, Alkaline phosphatise, Aminopeptidase, Lactoferrin, Translactoferin, IgM, MMP-13, MMP-8, MMP-9, Cathepsin B, Osteonectin, Osteocalcin, Osteopontin, Osteopontin, Elastase Platelet-activating factor, Epidermal growth factor, Platelet-derived growth factor, Esterase, Pyridinoline crosslinked carboxyterminal telopeptide, Fibronectin,sIgA (secretory IgA) Gelatinase, IgA, Trypsin, Vascular endothelial growth factor, IgG** | Cathepsin C gene Mutation, Collagen gene mutation, IL-1 polymorphisms, IL-10 polymorphisms, Tumor necrosis factor, Polymorphisms. | Aggregatibacter Actinomycetemcomitans, Campylobacter rectus, Mycoplasmas, Porphyromonas Gingivalis, Prevotella intermedia, Peptostreptococcus Micros, Prevotella nigrescens, Treponema denticola, Tannerella forsythia. Treponema socranskii. | Calcium, Cortisol, Hydrogen sulphide, Methylmercaptan, Methylmercaptan, Methylmercaptan, Pyridine. |

**Table. 1 Biomarkers For Periodontal Disease.**

| | Disease present | Disease Absent |
|---|---|---|
| **Test positive** | A (True-positive) | C (False-positive) |
| **Test negative** | B (False-negative) | D (True-negative) |

$$\text{Sensitivity} = \frac{A}{A+B} \qquad \text{Specificity} = \frac{D}{C+D}$$

$$\text{Positive predictive value} = \frac{A}{A+C}$$

$$\text{Negative predictive value} = \frac{D}{B+D}$$

Thus if a reliable predictive test or test kits were developed it could predict future periodontal activity and thus enable site specific treatment to be given before irreversible damage had occurred. For this to be the case the marker, as already stated above, must have been shown in human longitudinal studies to have highly statistically significant correlations with confirmed attachment loss, both at the predictive and the attachment loss times. It should also have very high positive and negative predictive values in diagnostic values.

Following are a few illustrations to understand it better; in patients who had clinical indicators of periodontitis salivary levels of TNFa were elevated[5], Mean levels of IL-1b and MMP-8 in saliva were significantly higher than control subjects[5]. Thus MMP-8 is not only an indicator of disease severity but also of

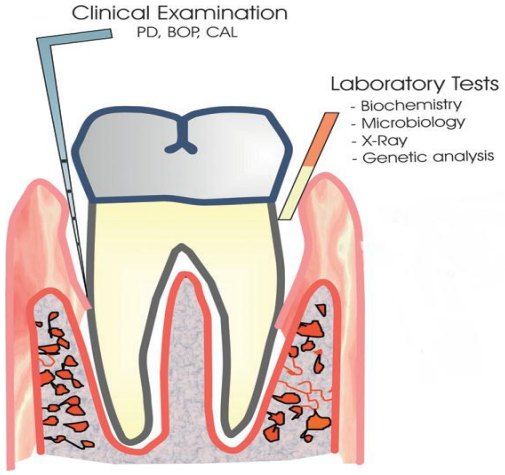


**Fig.1 Current and future diagnostic tools**

disease activity[5]. The level of C-reactive protein; a systemic biomarker is directly related to an individual's periodontal status[5] which can be tested by lab-on-chip method[6]. Periodontitis-prone individuals are capable of secreting high levels of salivary IgA[5].

Only Biomarkers with such credentials should be used in clinical practice and could be used for the following reasons:-

- Help to prevent destructive disease.
- Help to prevent progression of disease.
- Identify high risk patients.
- Target treatment to specific sites.
- Help to monitor periodontal treatment

Henceforth; we move onto biomarkers of periodontal disease activity available as Commercial diagnostic tests and those under development.[7] as enlisted below in Table. 2

| Assay | Kit | Manufacturer/ supplier | Availability | Comments | References |
|---|---|---|---|---|---|
| **Culture& biochemical Identification (GOLD STANDARD)** | Laboral | Laboral, France | Yes | Quantification/identification after bacterial culture of A. a,B. f, C. r, F. n, P. i, P. g, P. M aids in detection of proteinase , elastase | |
| | Prognostik | Dentsply | ?? | | |
| **Immunological dection (ELISA** | Evaluisite test | Kodak,Eastman company. (Switzerland) | No | Detects bacterial antigens of A.a, P. i, P. g. can be used at chairside. | (8) |
| **Bacterial enzymes** | Perioscan | Oral B laboratories OraTec Corporation | No | Detects enzymatic activity of Aa, Bf, Pg detects enzymatic activity of B. f, P. g, T. d | (9) |
| | BANA periodontal test | Manassas (USA) | Yes | | |
| **Bacterial toxins** | TOPAS | Affinity Labeling Technologies (USA) | Yes | Detects toxins derived from anaerobic metabolism and measures GCF protein level. | |
| **Host enzymes** | Periocheck | Collagenex pharmaceuticals | No | Detects enzymatic activity derived from GCF(Matrixmetalloproteinases and neutral protease enzymes) | (9) |
| **Nucleic.acid technology** | Affirm DP | Microprobe (USA) | ?? | DNA probes for A. a, B. f, P. i, P. g, T. D | (10) |
| | | | | DNA probes for A.a, C.r, E.c, F.n, P.i, P.g. | |
| | BTD test | BioTechnicaDiagnostic (USA) | ?? | DNA probes for B. f, P. g | |
| | OMTL test, | USC (USA) | Yes | DNA probes for A. a, P.i, P. g, E. c, F. n, T. d, C. r, B.f | |
| | Omnigene | Omnigene(USA) | Yes | DNA probe for b.f, P.g | (11) |
| | Periodontal microbial identification test. | Saigene corporation (USA) | ?? | DNA probes for A.a, P.g, P.i, E.c, B.f, C.r, T.d, F.n | (12) |
| | ANAWA, DMDx®/ Pathotec® | Switzerland | Yes | DNA probes for A.a, P.g, P.i, E.c, B.f, C.r rRNA quantification for P. g, A.a, B. f, T. d | |
| | Parogene | France | No | DNA probes for A.a, P.g, B.f, T.d. PCR detection for A.a, P.g, P.i, B.f, T.d | |
| | Explore | Explore (Netherlands) | Yes | Detection of A.a, P.g, P.i, P.d | (13) |
| | IAI Pado test 4.5 | IAI(Switzerland) | Yes | | |
| | Microdent® test | HAIN(Germany) & MicroDentex (USA) | Yes<br>Yes | | (14) |
| | MicroDent test kit | Szabo-scandic(Austria) | Yes | | |

A. a: Aggregatibacter actinomycetemcomitans, B. f: Bacteroides forsythus, C. r: Campylobacter rectus, E. c: Eikenella corrodens, P.m: Peptostreptococcus micros, P. g: Porphyromonas gingivalis, P. i: Prevotella intermedia, P. d: Prevotella denticola, T. d: Treponema denticola.

**Table. 2: Commercial diagnostic tests.**

There are some advantages of these systems over conventional diagnosis since few may be capable of detecting or predicting periodontal disease activity. There are also, however, some serious drawbacks of all these tests described below.

**Advantages:**

- Some; for eg. cathepsin B, elastase etc.. are predictive of disease activity in longitudinal studies.
- Simple to use, particularly the colour detection systems.
- Can be read after a short time.
- Can be shown to the patient and related to the tooth site.

**Disadvantages**

- The choice of the most appropriate biomarker may still be difficult at the present state of knowledge.
- There is difficulty in determining the sites to sample and when to sample them.
- They are not cost effective.
- If a moiety is associated with inflammation this may mask its association with destructive disease.

**Critical Evaluation**:

All of the markers used in the commercially available test systems and those under development have been shown to be capable of detecting disease activity. However, only a few of the enzymes described above appear to be actively predictive of disease activity (Fig. 2)(Chart 1).

Therefore the evaluation of some of the recently used biomarkers and some under developmental stage are shown below in Table 3.

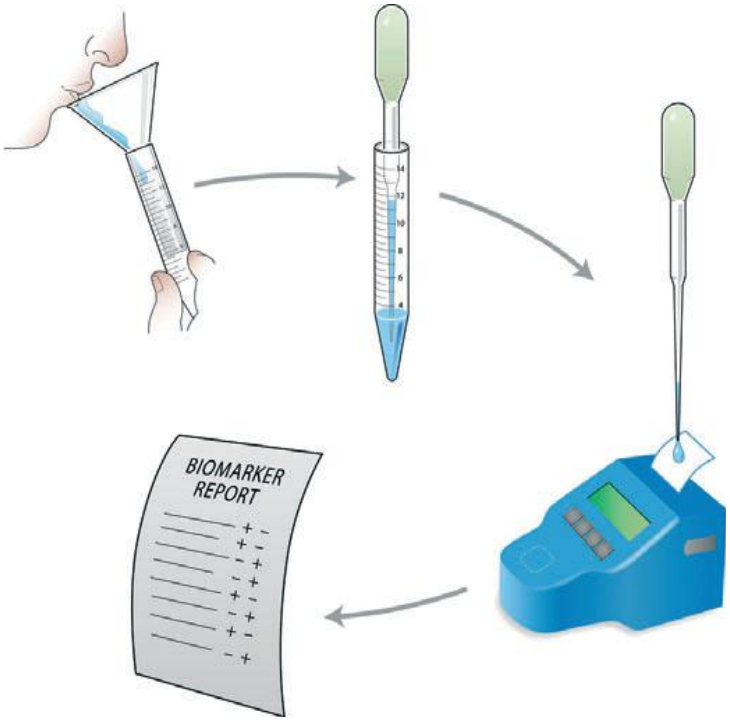


**Figure 2: Strategy for oral fluid sampling and analysis with a rapid point-of-care or lab-on-a-chip device for the generation of a periodontal disease biomarker report.**

| Diagnostic test | Disease active sites | Sites at Risk | Response to treatment | Tool for patient education |
|---|---|---|---|---|
| DNA analysis | ✓ | X | ✓ | ✓ |
| Immunological assays | ✓ | X | ✓ | X |
| Enzyme based assays | ✓ | X | ✓ | ✓ |
| GCF markers | ✓ | ? | ✓ | ✓ |

**Chart 1: Clinical Assesment Charting For Office Use**

| Sl no. | Assay. | Diagnostic kit. | Critical Evaluation. |
|---|---|---|---|
| 1 | Culture & biochemical Identification (GOLD STANDARD) | Laboral | It is highly technique sensitive & time consuming.<br>But it can be used for testing against resistant pathogens.<br>Pathogens of secondary importance can also reported if found in high percentage. |
| 2 | Bacterial enzymes & host enzymes | BANA(benzoyl-DL-arginine-β-napthylamide)<br><br>Periocheck<br>Perioscan | Pathogens like T. d, B. f, P. g can be detected. With 90-96% sensitivity, 83-92% specificity. But A. a can't be detected; using BANA.<br><br>Clinical studies have shown the usefulness of BANA hydrolytic activity, the presence of which is significantly correlated with pocket probing depths and attachment loss greater than 4 mm.<br>The number of BANA-positive sites observed in periodontitis patients decreases after successful therapy comprising scaling, root planing andadministration of systemic antibiotics.<br><br>Both periocheck & perioscan were not very successful & some are no longer distributed. |
| 3 | Immunological detection | Evalusite | Polyclonal & monoclonal antibodies are conjugated along with fluroscent reporters to enhance the specificity & sensitivity. Both are relatively low for this test.<br>Therefore this commercial product is not widely marketed. |
| 4 | Nucleic acid technology<br><br>Genomic probe.<br><br>Oligonucleotide probe. (CHUBA introduced them)<br><br>Chemiluminesence generating<br><br>Oligonucliotide probes | DMDx/PATHOTEK<br><br>IAI Pado Test 4.5 System<br><br>LCL®Periodontitis-Test | P. i, P. g can be detected with the aid of purified DNA fragments, but the same way A. a could not be detected.<br>Species such as P. i, P. g, & also A. a could be detected. Using radioactively labelled DNA probes.<br><br>It is a laboratory procedure for demonstrating periodontopathic bacteria.<br><br>Both IAI & LCL has been performing tests to check for its efficacy, & inclination towards it is rapidly growing in practitioners. |
| 5 | Biochemical Identification | Prognostik (Dentsply)<br><br>Pocket watch<br><br>Periogard ( Colgate) | Helps in detection of elastase & protinease in GCF<br>The commercial firms owning these tests are constantly changing because some of them sell the rights to their products to others.<br>For this reason, the firms cited as owning these tests may not remain accurate in the future.<br><br>Detects aspartate aminotransferase through colorimetric detection<br><br>Detects AST levels in GCF through colorimetric detection. |

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**Table 3. Available test kits and their evaluation.**

**Current Concepts & Future Trends:**

Therefore, now there is a whole new approach for the future use of oral fluids, especially in the field of diagnostics. A tremendous amount of research activity is currently under way to explore their role as a possible medium in a variety of applications. There are quite a few commercial diagnostic kits under practice such as β-glucuronidase: Abbott laboratories (U.S.A), dentoAnalyzer is among the first quantitative MMP-8 chair-side testing devices in periodontal and peri-implant diagnostics and research. Immunofluorometric assay and dento-analyzer can detect MMP-8 from GCF samples. Electrochemical biosensors coupled to Magnetic Beads are also used for the Detection of Clinical Biomarkers. Also several researchers have focused on genetic single nucleotide polymorphisms in the study of periodontitis. There is a genetic susceptibility test currently available for severe chronic periodontitis (Interleukin Genetics, Waltham. Massachusetts). Individuals identified as "genotype positive," are more likely to have the phenotype of overexpression of this gene. In this way genomics has been found to be applicable in the prediction of predisposition to periodontitis in certain patient populations. Moreover, technologies such as nucleic acid and protein microarrays and microfluidics are under development for risk assessment and comprehensive screening of biomarkers. These recent advances are leading to the development of more powerful diagnostic tools for practitioners to optimize their treatment predictability.

**Conclusion**

Thus, it can be concluded that though several products show potential benefit; which gives a clue as to which tissue components are at risk, most of the test kit, or biomarkers yield little or no additional information, at high costing. It is also clear that no single marker has been able to fulfil all the criteria necessary for assessment of the clinical state of the periodontium, and future research should be directed possibly at the production of "marker packages" As of now various efforts are on to develop an ideal test, but actual use as a chairside diagnostic is still illusive. Therefore the development of a wide spectrum of markers is the primary goal of periodontal research.

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**References:**

1) Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69(3):89–95
2) Taba M, kinney J, Kim A, Ginnobile W. Diagnostic Biomarkers for Oral and Periodontal disease. Dent Clin N Am 49 (2005) 551–571.
3) Chapple I. Periodontal diagnosis and treatment — where does the future lie? Periodontology 2000; 2009: 51:9-24.
4) Armitage GC. Periodontal diagnoses and classification of periodontal disease. Periodontol 2000 2004; 34: 9-21.
5) Zhang L et al. The clinical value of salivary biomarkers for periodontal disease. Periodontology 2000; 2009: 51:25-37.
6) Giannobile W, Beikler T, Kinney J et. al Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontology 2000; 2009: 50: 52-64
7) G. Suchett-Kaye et al. Clinical usefulness of microbiological diagnostic tools in the management of periodontal disease Res. Microbiol. 152 (2001) 631–639
8) Boyer B.P., Ryerson C.C., Reynolds H.S., Zambon J.J., Genco R.J., Snyder B., Colonization by Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia in adult periodontitis patients as detected by the antibody-based Evalusite Test, J. Clin. Periodontol. 23 (1996) 477–484
9) Hemming K.W., Griffiths G.S., Bulman J.S., Detection of neutral protease (Periocheck) and BANA hydrolase (Perioscan) compared with traditional clinical methods of diagnosis and monitoring of chronic inflammatory periodontal disease, J. Clin. Periodontol. 24 (1997) 110–114
10) Schlegel-Bregenzer B., Persson R.E., Lukehart S., Braham P., Oswald T., Persson G.R., Clinical and microbial findings in ederly subjects with gingivitis or periodontitis, J. Clin. Periodontol. 25 (1998) 897–907.
11) Shiloah J., Patters M.R., Waring M.B., The prevalence of pathogenic periodontal microflora in healthy young adult smokers, J. Periodontol. 71 (2000) 562–567.
12) Tanner A.C.R., Maiden M.F.J., Zambon J.J., Thoren G.S., Kent R.L. Jr., Rapid chairside DNA probe assay of Bacteroides forsythus and Porphyromonas gingivalis, J. Periodont. Res. 33 (1998) 105–117.
13) Mättö J., Saarela M., von Troll-Lindén B., Jousimies- Somer H., Torkko H., Alaluusa S., Asikainen S. Distribution and genetic analysis of oral Prevotella intermedia and Prevotella nigrescens, Oral. Microbiol. Immunol. 11 (1996) 96–102.

14) Kornman K.S., Newman M.G., Alvarado R., Flemmig T.F., Nachnani S., Tumbusch J., Clinical and microbiological patterns of adults with periodontitis, J. Periodontol. 62 (1991) 634–642.
15) Conrads G. Testing for marker Bacteria in progressive periodontitis: The Europian Experience. Infectious Diseases in Clinical Practice, 2001;10:481–487
16) Sorsa T. Herna´ ndez M., Leppilahti J., Netuschi L., Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. Oral Diseases (2010) 16, 39–45